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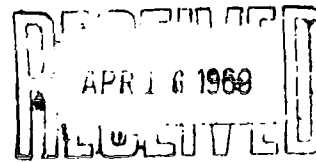
METHYL BROMIDE AS AN AID
TO ETHYLENE OXIDE STERILIZATION

Dorothy M. Partner

David R. Spiner

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MARCH 1969 D C



DEPARTMENT OF THE ARMY

Fort Detrick

Frederick, Maryland

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**DEPARTMENT OF THE ARMY
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Frederick, Maryland 21701**

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METHYL BROMIDE AS AN AID TO ETHYLENE OXIDE STERILIZATION

Dorothy M. Portner

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**Physical Defense Division
COMMODITY DEVELOPMENT & ENGINEERING LABORATORIES**

Project 1B662706A072

March 1969

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ABSTRACT

The sporicidal activity of ethylene oxide (EtO), methyl bromide (MeBr), and a mixture of EtO and MeBr was determined against B. subtilis var. niger spores on cloth patches either uncovered or sealed within polyethylene or polyvinyl chloride plastic. No difference in activity between the mixture and EtO alone was observed against spores on cloth not protected by plastic. However, the mixture was (i) measurably more effective than EtO alone against spores sealed within polyvinyl chloride and (ii) possibly slightly more effective against spores sealed in polyethylene. The bactericidal activity of MeBr alone was considerably less than that of EtO alone, and the individual activity of both was curtailed by the plastics. MeBr apparently does not have a synergistic effect upon the rate of EtO sterilization, but rather promotes the rate of EtO penetration, at least through some plastics.

I. INTRODUCTION*

At the Seventh COSPAR International Space Science Symposium, the Russian scientists Vashkov and Prishchep¹ stated that planetary spacecraft could be sterilized with a gaseous mixture containing 60% ethylene oxide (EtO) and 40% methyl bromide (MeBr). From their data one cannot determine whether the mixture of the two has a synergistic effect or whether penetration is accelerated. The National Aeronautics and Space Administration evinced an interest in studying the activity of this mixture because EtO gas is being considered in a phase of their sterilization procedure for interplanetary space probes.

Numerous studies and practical applications of EtO gaseous sterilization are described in recent reviews,²⁻⁴ but only a few papers^{1,5-7} discuss its use with MeBr. The lack of precise information on the bactericidal activity of MeBr with EtO as compared with activity of EtO alone motivated this investigation. The effectiveness of EtO, MeBr, and the EtO-MeBr mixture to penetrate and sterilize was determined on spore-contaminated cloth sealed within polyethylene or polyvinyl chloride plastic.

II. MATERIALS AND METHODS

A. PREPARATION OF TEST SAMPLES

An aqueous stock spore suspension of Bacillus subtilis var. niger was used to contaminate cotton patches (5/8-inch diameter). Each contaminated patch, containing 4×10^5 spores, was conditioned for 4 days at 25 C and 53% RH by suspending the patch over a saturated nickel chloride solution in a stoppered bottle. Patches were conditioned to avoid the adverse effect of desiccation shown by Gilbert et al.,⁸ in their EtO studies. To study the effect of gaseous penetration, each conditioned patch was placed in a small bag of polyethylene (6 mils thick) or polyvinyl chloride (12.5 mils thick) that was heat-sealed just before the gaseous treatment. Half of the polyvinyl chloride bags were then heat-sealed each within a second bag. For comparable tests with polyethylene, contaminated patches were sealed within two or four bags to give 12 and 24 mils thickness, respectively.

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B. EXPOSURE CHAMBER

Three vacuum-type desiccators with modified tops (Fig. 1) were used at 25 C for exposures to EtO-MeBr, EtO, and MeBr. Two each of uncovered patches and patches protected by both 12- or 24-mil-thick plastic were placed in each desiccator. To insure that adequate moisture was available for sterilization, the spore-contaminated patches were preconditioned at 53% RH before sealing in plastic, and water (0.2 ml on filter paper) was added to the exposure chamber before the gases were introduced.

For the EtO-MeBr mixture, a vacuum was drawn on the chamber to reduce the pressure to 302 mm Hg. The chamber was returned to atmospheric pressure by admitting 270 mm Hg of pure EtO and 188 mm Hg of MeBr. This ratio of 60% EtO (656 mg/liter) and 40% MeBr (944 mg/liter) (v/v) was the same as that used by Vashkov and Prishchep.¹ For comparable conditions, the same vacuum was drawn when either EtO or MeBr was used alone, but air was substituted for the other gas. Two replicate tests with 2-, 4-, and 8-hour exposures were conducted with both the individual gases and the mixture, all at 25 C.

C. METHOD OF ASSAY

Immediately after the gaseous treatment, the sealed plastic bags were cut with sterile scissors and the patches were transferred to sterile stoppered test tubes containing 10 ml of water, as were the patches not protected by plastic. The tubes were shaken vigorously, and then one 5-ml and two 1-ml undiluted portions, as well as duplicate serial dilutions, were plated in trypticase soy agar. Plate counts were made after 72 hours of incubation at 32 C. Contaminated patches conditioned but not exposed to a gaseous treatment were also assayed to serve as a basis for computing per cent recovery.

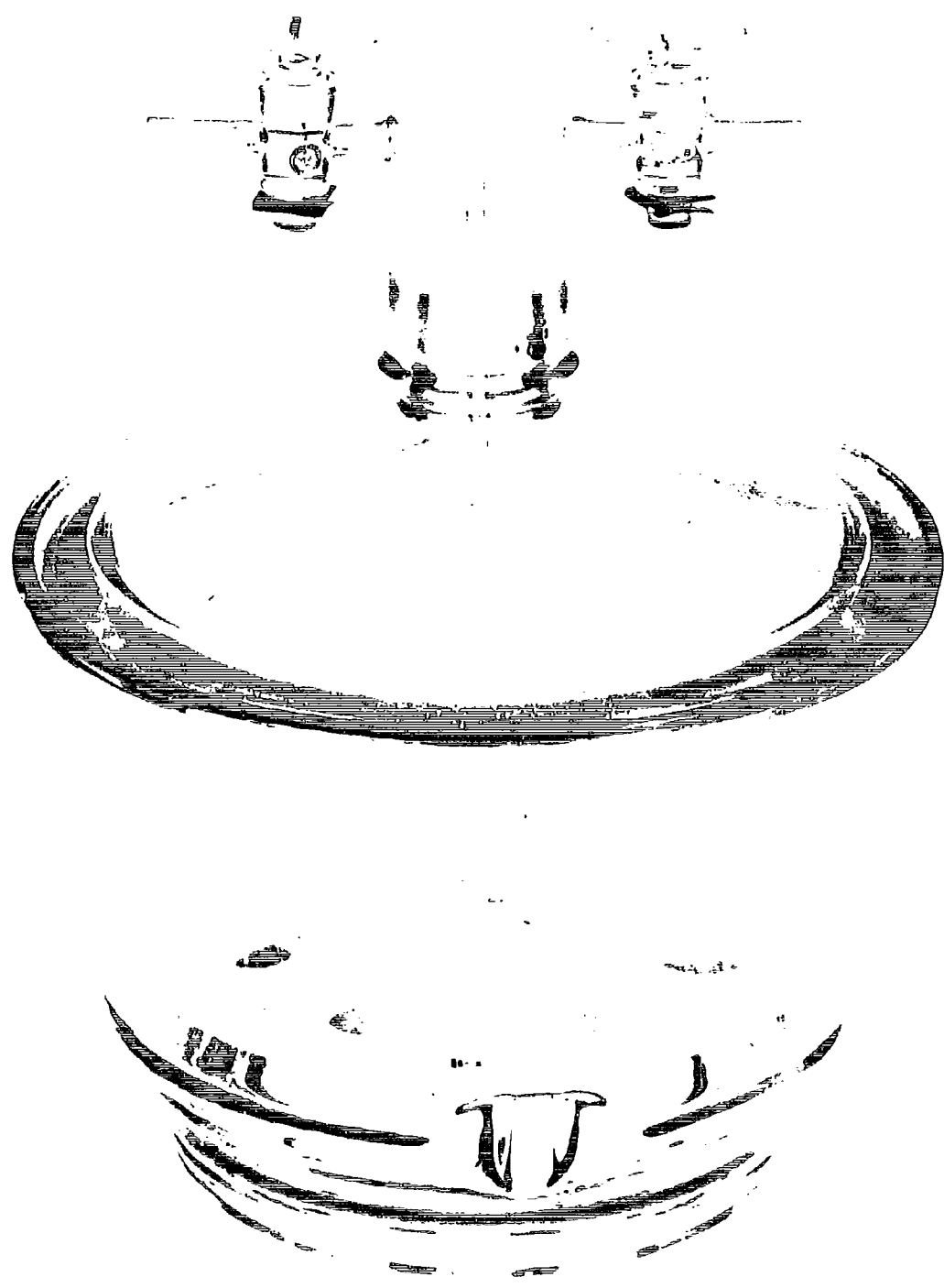


FIGURE 1. Exposure Chamber. (ID Rev. C-6012)

III. RESULTS AND DISCUSSION

The results obtained for uncovered patches and those protected by 12-mil-thick plastic are given in Table 1 for ready comparison, while the data for the 24-mil-thick plastic, which indicate a more definite trend, are presented graphically (Fig. 2). There was no statistically significant difference between the activity of the EtO-MeBr mixture and EtO alone against spores on uncovered patches. With both types of plastic, however, the recovery after exposure to the EtO-MeBr mixture was significantly lower than the recovery after exposure to EtO alone for 4 but not for 2 hours. The magnitude of the difference was large with polyvinyl chloride but not of practical importance with polyethylene. The increased penetration rate of EtO through plastic is apparently due to the effect of MeBr. Figure 2 illustrates this clearly; EtO requires about twice as long to penetrate 24-mil-thick polyvinyl chloride film if MeBr is not present. After penetration, the slopes, if well defined, probably would be parallel, indicating the same rate of kill for both the mixture and EtO alone.

The death rate of unprotected spores exposed to MeBr alone was very low (Table 1). In the 12-mil-thick polyethylene, some spore reduction occurred after 8 hours' exposure to MeBr alone, but in 24-mil-thick polyethylene and in 12- and 24-mil-thick polyvinyl chloride, the spore reduction was not appreciable. The observations that (i) the plastic restricted the penetration of MeBr and significantly slowed the death rate, (ii) MeBr did not affect the death rate of EtO on unprotected spores, and (iii) the death rate with EtO for spores within polyvinyl chloride was accelerated by the MeBr suggested that MeBr was dissolving in and being retained by the plastic, thus acting as a vehicle for EtO penetration. To determine whether MeBr need only be present in the plastic at the time of the EtO treatment, polyvinyl chloride plastic (12.5 mils thick) bags were sealed and exposed to MeBr for 2 hours. After that, each MeBr-treated plastic bag was cut open and a contaminated cloth patch was inserted and sealed within the bag and immediately exposed to EtO alone for 2 hours. The results showed that the decrease in spore recovery after a 2-hour exposure to EtO was about fivefold greater with MeBr-treated plastic than with untreated plastic.

The penetration of EtO through materials, such as polyvinyl chloride plastic, dry gelatin,⁶ and baled jute bags,⁷ appears to be increased by the addition of MeBr, thus shortening the exposure period required for sterilization. Vashkov and Prishchep¹ reported that MeBr enhanced the activity of EtO through various materials, as well as directly on exposed cloth patches. This study confirms the report that MeBr can assist penetration of EtO through some materials. However, no general statement can be made regarding penetration of the mixture through the many plastics and materials used in spacecraft construction. This increased permeability, not a synergistic action, seems to account for those instances in which organisms behind protective barriers are killed more rapidly by the mixture than by EtO alone. On unprotected organisms, the addition of MeBr does not measurably increase the kill caused by EtO alone.

TABLE 1. EFFECTIVENESS OF EtO, MeBr AND AN EtO-MeBr MIXTURE ON B. SUBTILIS VAR. NIGER SPORES
ON CLOTH PATCHES EITHER UNPROTECTED OR ENVELOPED IN POLYETHYLENE
OR POLYVINYL CHLORIDE PLASTIC BAGS^{a/}

Exposure, hours	Recovery, % ^{b/}							
	No Plastic		MeBr		Polyethylene ^{c/}		Polyvinyl Chloride ^{c/}	
	EtO-MeBr	EtO	EtO	MeBr	EtO-MeBr	EtO	EtO-MeBr	MeBr
1	0.059	0.019	-d/	-	-	-	-	-
2	0.00073	0.00009	18	3.7	12	49	0.052	5.5
4	0.00001 ^{e/}	0	3.9	0	0.0018	16	0	0.41
8	0.00003 ^{e/}	0	0.65	0	0	2.4	0	21

a. EtO = ethylene oxide; MeBr = methyl bromide.

b. Data for No Plastic are means of six to 26 tests; all other data are means of four tests.

c. Plastic about 12 mils thick.

d. Not tested.

e. Equivalent to one colony recovered from a number of samples.

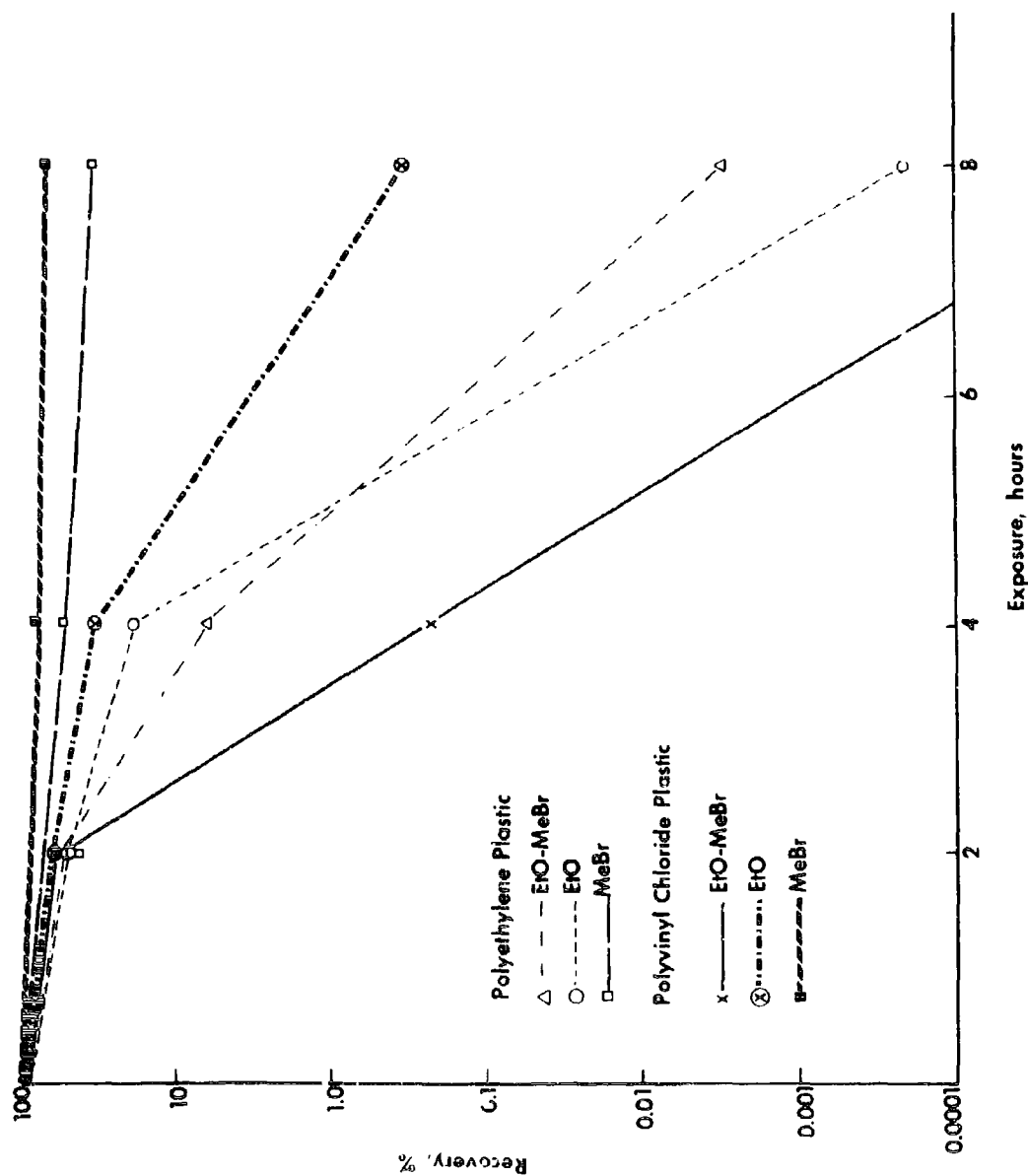


FIGURE 2. Comparison of the Activity of EtO-MeBr, EtO, and MeBr Against Spores Protected by 24-mil-Thick Plastic.

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13. ABSTRACT		
<p>The sporicidal activity of ethylene oxide (EtO), methyl bromide (MeBr), and a mixture of EtO and MeBr was determined against <u>B. subtilis</u> var. <u>niger</u> spores on cloth patches either uncovered or sealed within polyethylene or polyvinyl chloride plastic. No difference in activity between the mixture and EtO alone was observed against spores on cloth not protected by plastic. However, the mixture was (i) measurably more effective than EtO alone against spores sealed within polyvinyl chloride and (ii) possibly slightly more effective against spores sealed in polyethylene. The bactericidal activity of MeBr alone was considerably less than that of EtO alone, and the individual activity of both was curtailed by the plastics. MeBr apparently does not have a synergistic effect upon the rate of EtO sterilization, but rather promotes the rate of EtO penetration, at least through some plastics.</p>		
14. Key Words		
<p>*Methyl bromide *Ethylene oxide *Sterilization Decontaminants Decontamination <u>Bacillus subtilis</u> var. <u>niger</u> Polyethylene Plastics</p>		

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